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Final Report

CRADA with Implant Innovations, Inc., and Pacific Northwest National Laboratory (PNL-137):

Bioactive and Porous Metal Coatings For Improved Tissue Regeneration

A.A. Campbell

January 2000

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FINAL REPORT

Bioactive and Porous Metal Coatings for Improved Tissue Regeneration

Allison A. Campbell, Lin Song, Shari Li and Marisol Avila (AWU)

Specific Project Objectives:

The goal of this project was to develop methods for producing bioactive and porous metal coatings that overcome many of the problems associated with currently available implants.

Specifically, the goals of the project are to:

1.0 Develop the Surface-induced Mineralization (SIM) process for the deposition of calcium phosphate on medical alloys
- 1.1 Demonstrate surface treatment with self-assembling monolayers (SAMs) on medical alloys
- 1.2 Optimize mineral deposition process
- 1.3 Characterize coating

2.0 Produce and coat Void Metal Composite (VMC) titanium alloy materials
- 2.1 Coat existing VMC materials
- 2.2 Produce new titanium VMC structures

3.0 Incorporate bone morphogenic proteins into the calcium phosphate coating.
- 3.1 Quantify protein uptake/release

4.0 Biological testing of coatings
- 4.1 Conduct *in vitro* evaluation of cellular response to coatings
- 4.2 Conduct *in vivo* histological and biomechanical evaluation

5.0 Demonstrate use of ZrPdRu alloy
- 5.1 Synthesize alloy with specific stoichiometry
- 5.2 Demonstrate the ability to coat a solid substrate with a bioactive coating
- 5.3 Produce a VMC structure

Summary of Research Activities:

Our first objective was to develop the SIM process for the deposition of calcium phosphate films. This process is based on the observation that, in nature, living organisms use macromolecules to control the nucleation and growth of mineral phases. These macromolecules act as templates where various charged functional groups, contained within the molecule, can interact with the ions in the surrounding media, thus stimulating crystal nucleation and growth. Rather than using complex proteins or biopolymers, surface modification schemes were developed to place simple functional groups on the underlying substrate using self-assembling monolayers.

Once the substrate was chemically modified, it was then placed into an aqueous solution containing soluble precursors of the desired mineral coating. Solution pH, ionic concentration and temperature is maintained in a regime where the solution is supersaturated with respect to the desired mineral phase, thereby creating the driving force for nucleation and growth. Typical coatings are shown in figure 1.

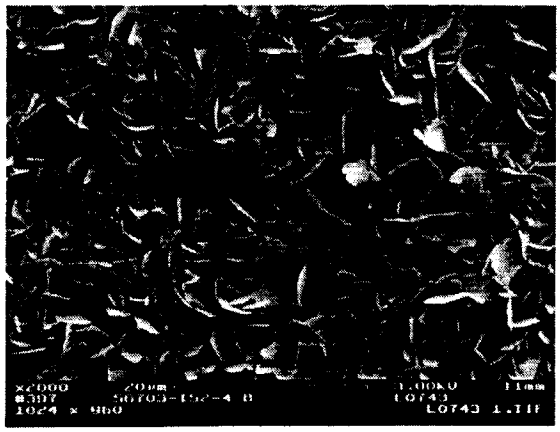

Figure 1

Our next objective was to demonstrate the ability to coat the pores and surfaces of existing VMC materials. Again, SAM formation was established on the VMC surface and the materials were successfully coated using the newly established solution parameters as shown in figure 2.

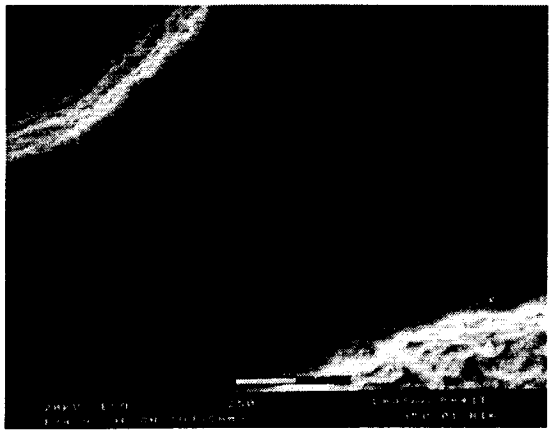

Figure 2

Another focus has been on the incorporation of bone morphogenic proteins and other therapeutic agents into the calcium phosphate coating. Initial experiments were done on bovine serum albumin (BSA) in order to determine if the presence of protein within the coating or the supersaturated solution would interfere with the SIM process. BSA,

transforming growth factor-β, 5–flourouracil and chlorhexidine were all incorporated into the coatings.

Finally, biological testing of coatings has been performed. We have been working with Dr. John E. Davies at the Centre for Biomaterials, University of Toronto. He has developed an *in vitro* model system for the evaluation of the interaction of osteoclasts and osteoblasts implant surfaces. Using his system, we evaluated the SIM coatings and showed that the coatings supported bone formation as shown in figure 3.

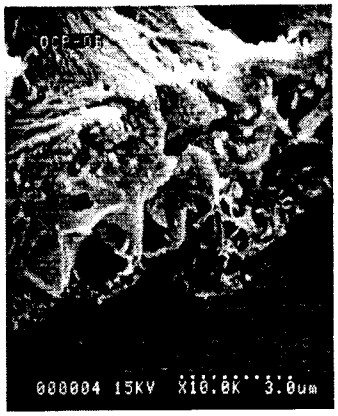

Figure 3

Summary of accomplishments:

1) Developed and optimized the SIM process for the deposition of calcium phosphate films. First, SAM formation on Ti 6,4 was characterized using Auger Spectroscopy (AES) and solid state CP MAS ^{13}C and ^{29}Si NMR. Once SAM formation was verified, different solution chemistry parameters were explored. Criteria for selecting optimum conditions were the time of deposition and coating quality (i.e., uniformity, thickness). Initial work focused on various calcium and phosphate concentrations and adjusting the solution pH to 7.4 using KOH. While several of the solution conditions tried produced adequate coatings, the solution preparation process was rather delicate. We then tried controlling solution pH via the decomposition of urea. While the solution prep was simpler, the resulting films were discontinuous after 6 cycles in fresh supersaturated solutions. We have recently gone to a much simpler system in which the concentrations of KH_2PO_4 and Na_2HPO_4 are adjusted to yield the desired pH and $CaCl_2$ is then added to create a supersaturated solution. This new solution results in 0.5 μm thick octacalcium phosphate (OCP) coatings after1 hour of exposure. The resulting crystallites also have a much smaller grain size than those resulting from the urea-based solution.

2) Coated existing cylindrical VMC titanium alloy materials and demonstrated uniform mineral formation within the pore structure. Figure 2 shows a VMC piece after 6 cycles in a supersaturated solution. In was quite clear that the mineral has formed uniformly down into the pores of the VMC.

3) The incorporation of bone morphogenic proteins has been demonstrated. Initially bovine serum albumin was investigated because of its availability and ease of identification. Two different processes were attempted. The first involved the addition of BSA directly to the supersaturated solution. The second method was a two step process in which one layer of mineral was deposited followed by exposure to a separate solution containing the protein then back to another calcium phosphate solution. The amount of BSA incorporated into the coating after 6 cycles was determined to be 0.2% by weight.

4) We have demonstrated *in vitro* both ostoeclast and osteoblast activation by the mineralized OCP coating. The deposition of new bone by osteoblasts was observed after 9 and 12 days. In a separate experiment, osteoclasts resorption pits were observed in the coating after 2 weeks.

5) We have successfully converted the octacalcium phosphate coating to hydroxyapatite using a heat treatment (200°C) subsequent to the aqueous deposition. We are currently looking at alternative methods for producing HAP coatings as well as investigating the *in vitro* response of these HAP coatings.

6) In vivo animal testing has been performed and preliminary results indicate that the coatings facilitated bone formation.

Tangible Outcomes
Published papers emanating from this project:

AA Campbell, L Song, X Li, C Bottoni, BJ Nelson, DE Brooks, and ES DeJong, *Development, Characterization, and Anti-Microbial Efficacy of Hydroxyapatite-Chlorhexidine Coatings Produced by Surface Induced Mineralization*, submitted to Journal of Biomedical Materials Research, Applied Biomaterials, 9/99.

AA Campbell, GE Fryxell, JC Linehan and GL Graff, *Surface-Induced Mineralization: A New Method for Producing Calcium Phosphate Coatings*, J. Biomed. Mater. Res., **32**(1), 111-118, (1996).

L Song, AA Campbell, XS Li and BC Bunker, *Surface Induced Calcium Phosphate Nucleation and Growth*, MRS Symp. Proc., **414**, 35-41, (1996).

AA Campbell, GL Graff, L Song and KR Sump, *Bioactive Void Metal Composites for Orthopedic Implant Devices*, MRS Symp. Proc., **414**, 177-182, (1996).

DL Wheeler, AA Campbell, GL Graff and GJ Miller, *Histological and Biomedical Evaluation of Calcium Phosphate Coatings Applied through Surface-induced Mineralization (SIM) to Porous Titanium Implants*, accepted to J. Biomed. Mat. Res., (1996).

AA Campbell, BC Bunker, GE Fryxell, GL Graff, M Avila, GJ Miller, and DL Wheeler, *Surface Induced Mineralization of Bioceramic Coatings for Orthopedic Implants*, Fourth Euro Ceramics, Vol. **8**, 155-160, (1995).

Presentations at National and International Meetings

AA Campbell, L Song, X Li and M Avila, *Biomimetic Materials for the Delivery of Therapeutic Agents*, **invited talk**, American Chemical Society National Meeting, Anaheim, March, 1999.

AA Campbell, *Metallic and Ceramic Coatings, Including Biomimetic Coatings for Biomedical Applications,* **invited talk**, International Conference on Metallurgical Coatings and Films, San Diego, CA April, 1998.

AA Campbell, L Song, X Li XY Shen and JE Davies, *Osteoclast Resorption and Bone Growth on Octacalcium Phosphate Films In Vitro*, 30th Annual IMS Convention, Seattle, WA, July, 1997.

AA Campbell, L Song, X Li XY Shen and JE Davies, *Osteoclast Resorption of, and Bone Growth on, Octacalcium Phosphate Films In Vitro*, **invited talk**, Cells and Materials National Meeting, Chicago, IL, May, 1997.

AA Campbell, L Song, GL Graff, GE Fryxell and JE Davies, *Enhanced Implant Stabilization*, ACers Basic Sciences Meeting, San Antonio, TX, October, 1996.

AA Campbell, L Song and KR Sump, *Bioactive and Porous Metal Coatings for Orthopedic Implants*, **invited talk**, Biomineralization Gordon Conference, Plymouth, NH, August, 1996.

AA Campbell, L Song, GL Graff, GE Fryxell, and KW Sump, *Bioactive and Porous Metal Calcium Phosphate Coatings for Improved Bone Regeneration*, 5th World Biomaterials Congress, May, 1996.

AA Campbell, GL Graff, L Song, and KR Sump, *Bioactive Void Metal Composites for Orthopedic Implant Devices*, Materials Research Society Fall Meeting, Boston, November, 1995.

AA Campbell, GE Fryxell, GL Graff, L Song, K Sump, GJ Miller, and DL Wheeler, *Bioactive and Porous Metal Coatings for Orthopedic Implants*, **invited talk**, Gordon Research Conference on Biocompatability and Biomaterials, July, 1995

AA Campbell, GL Graff, GJ Miller, and DL Wheeler, *Surface Induced Deposition of Calcium Phosphate Coatings for Orthopedic Implants*, Society for Biomaterials Foundation, Scottsdale, September, 1994.

AA Campbell and GL Graff, *Low temperature Solution Deposition of Calcium Phosphate Coatings for Orthopedic Implants*, American Ceramic Society Annual Meeting, Indianapolis, April, 1994.

Intellectual Property
Patent 5,958,430: AA Campbell and L Song, *thin Film Composition with Biological Substance and Method of Making*, issued 9/28/99. In appendix

Encountered problems
We were been unable to produce new VMC materials because the High Energy Rate Forming (HERF) equipment could not be salvaged from existing components. Work with the Pd alloy was never started since VMC processing could not be realized.

DOE/Laboratory Benefits
One main benefit to DOE has been the application of fundamental science funded from the Office of Basic Energy Sciences in the area of biomimetic synthesis of ceramic thin films to real work problems. Via the CRADA mechanism, it was possible for this work to be directed and refined to the specific problem of implant biocompatiblity. This demonstrates the ability to "put science to work".

Industrial Benefits Realized
Industrial benefits have not yet been fully realized. In order for this technology to be a commercial product, FDA approval much be obtained. However, once done, this technology can significantly enhance the products offered by Implant Innovations, Inc.

Appendices
Copies if the papers published under this work as well as a copy of the newly issued patent are enclosed.

BIOACTIVE VOID METAL COMPOSITES FOR ORTHOPEDIC IMPLANT DEVICES

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*Pacific Northwest National Laboratory is operated for the U.S. Department of Energy by Battelle Memorial Institute under contract DE-AC06-76RLO 1830 .

Abstract

Although significant advances have been made to provide mechanically strong and non-toxic metals and alloys, biological integration of devices into natural tissues remains a problem. The Surface Induced Mineralization (SIM) and Void Metal Composite (VMC) processes produce a bioactive porous metal implant coating/device which may address many of the problems associated with conventional processing methods.

The VMC process produces materials which have cylindrical pores of uniform diameter which can completely penetrate the structure of the material. The pore diameter, orientation and interconnectivety are easily controlled

The SIM process uses the idea of nature's template-mediated mineralization by chemically modifying the implant to produce a surface which induces heterogeneous nucleation from aqueous solution. SIM produced bioactive coatings provide 1) control of the thickness and density of the mineral phase, 2) a way to coat porous metals, complex shapes and large objects, 3) the ability to coat a wide variety of materials, 4) potential choice for the phase of the mineral formed.

Introduction

A number of nearly twenty million Americans and more than fifteen million others who are afflicted with degenerative bone and joint diseases as well as trauma victims who have suffered bone fractures need devices which anchor to the unaffected bony tissue around the defect site (1). Although significant advances have been made in the field of metallurgy to provide mechanically strong and non-toxic metals and alloys, biological integration of devices into natural tissues remains a problem (2-3). Thus many effective devices become loose over time and necessitate subsequent surgery to remove and replace the old device, a process fraught with high morbidity and mortality. Efforts to solve the problems associated with device anchoring have been highly fragmented among the biological, mechanical and surgical disciplines.

The high loads required of many implants restricts the selection of many materials. In addition to being biocompatible, the material must possess adequate fracture and fatigue resistance. While metals or metal alloys meet many of the biomechanical requirements, they have poor or nonexistent interfacial bonding between the metallic surface and the surrounding bone. In order to alleviate this problem, porous metal coatings have been applied to many implant surfaces. This facilitates bone ingrowth into the porous layer thus improving fixation due to improved mechanical interlock. However, there remains concern about poor interfacial bonding between the porous metal coating and the surrounding bone.

Many of the disadvantages of metallic implant devices can be diminished by the use of bioactive materials or coatings on the implant surface. Ducheyne (4) demonstrated that

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hydroxyapatite (HAP) surface coatings increased the rate of bone formation within a porous metal sample. In addition, HAP surfaces do not form fibrous-tissues but form an extremely thin, epitaxial bonding layer with existing bone (5).

Though there are many desirable features of bioactive surface coatings, an optimal technique for coating application has not been developed. Lacefield (6) found plasma coating formed a dense, adherent coating of HAP on a metal substrates, but cautioned that the coating of complex implant devices containing internal cavities was not feasible. More serious was the formation of an amorphous calcium phosphate in the film rather than HAP in more than 50% of the coating attempts (7).

Materials and Methods

Void Metal Composites (VMC)

The VMC process produces coatings that are open-celled with interconnecting porosity and the pore density can be controlled. "Wire VMC" (WVMC), has cylindrical pores of uniform diameter which may completely penetrate the structure of the material. The pores were formed with stacked pieces of brass mesh. A fine powder of the metal or alloy of the final structure, e.g. Ti-6Al-4V alloy, was packed by high frequency vibration into the interstices of the wire mesh. High speed compaction of the mixture, at elevated temperatures, produced a compact that had high density and good forming characteristics. The compact was then machined into its final shape. The brass mesh was removed from the densified material by dissolution in 6 N nitric acid. A final high temperature vacuum treatment (sintering) caused the metal structure around the spherical pores to fully densify, thereby achieving its optimum mechanical properties.

Surface Functionalization

The SIM process for the deposition of mineral phase onto various substrates has also been developed at Pacific Northwest national Laboratory (PNNL). In this work, surface modification of titanium and titanium alloys was accomplished using self-assembled monolayers (SAMs) (Figure 1) (8-10).

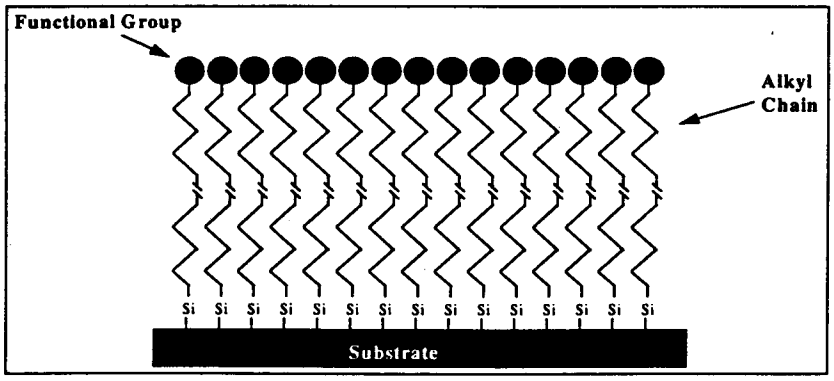

Figure 1. Self-assembled monolayer. The SAM molecule consists of three parts a) a silane coupling agent, b) the alkyl chain, and c) the functionalized end group (examples include -COOH, $-SO_3H$, $-PO_4H_2$, $-CH_3$, $-NH_2$).

The SAMs were attached to Ti-6Al-4V alloy wafers (polished on one side with a uniform oxide layer of 100 Å) as follows. Prior to SAM formation, the wafers were cut, placed into Teflon racks, and ultra sonicated 2-3 minutes in chloroform (Aldrich). Residual trace amounts of organic contamination was then removed by exposure to an air plasma for 10 minutes. Hydroxylation of the wafers was performed by treatment with 0.1 M KOH solution for several minutes. The resulting hydroxides were then protonated by immersing the wafers in 0.1 M HNO3 solution for 10 minutes. Following the acid soak, the wafers were thoroughly washed with deionized water and blown dry with a stream of dry nitrogen gas.

SAM formation was accomplished by placing the wafers in a 1% silane:cyclohexane solution for 30 minutes. Following SAM formation, the wafers were rinsed in 2-propanol (Aldrich) in order to remove any residual silane. Finally, the wafers were sonicated in chloroform for 5 minutes to produce mirror bright surfaces.

The terminus vinyl group of the alkylsilane tether was subsequently modified to sulfonic acid by exposure of the derivatized wafer to SO_3 gas in a reaction vessel for 1 minute. Following sulfonation, the wafers were removed, sonicated 10 minutes in deionized water, and blown dry with nitrogen gas.

Calcium Phosphate Mineralization

Solutions prepared using reagent grade chemicals (Fisher Scientific) and deionized, reverse osmosis (Millipore) CO_2-free water were filtered before use. Calcium phosphate deposition experiments were carried out in sealable glass containers. Supersaturated solutions were prepared by the slow addition of dihydrogen potassium phosphate to a calcium chloride solution. The pH was adjusted by the addition of 0.01 M KOH solution. Following solution preparation, a rack containing the derivatized SAM surfaces was placed into the supersaturated solution, the vessel was sealed, maintained at room temperatures, and gently stirred for the duration of the experiment. Following the desired reaction time, the substrates were removed from the mineralizing solution, rinsed with deionized water and blown dry with N_2 gas. Samples were then analyzed by scanning electron microscopy (Electroscan), energy dispersive X-ray analysis (Link Analytical), and X-ray diffraction (Phillips).

Results

Figure 2 shows a cross section of a VMC porous material. Clearly the pores are interconnected and oriented with respect to the surface of the material. In the WVMC, the pores can be made to lie in the plane of maximum desired ingrowth by appropriate orientation of the implant with a plane of maximum pore density in the bulk material.

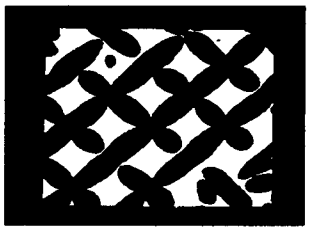

Figure 2. Optical micrograph of a VMC cross section.

The pore diameter can be controlled and is uniform throughout. Pore interconnectivity is retained as well.
Thus, it may be possible to create an implant surface which has channels that maximize bone ingrowth. Also, the interconnectivity of the pores will enable bone ultimately to grow to bone, lead to an increased mechanical fixation of the implant.

SIM produced bioactive coatings provide 1) the ability to control the thickness and density of the deposited mineral phase, 2) a superior and uniform adhesion to prosthesis surfaces, 3) an easy way to coat porous metals, complex shapes and large objects after the device has been produced, 4) the ability to coat a wide variety of metals, alloys, ceramics and plastics, and 5) potential choice for the type of mineral formed which may be further transformed when placed in the body.

Figure 3 shows the solubility isotherm for these phases as well as the range of solution conditions for the mineralization experiments. As an initial selection, solution concentrations ranging from pH 5 to pH 8 and a -log (Ca x PO_4) of 5 to 8 where chosen. The shaded region indicates solutions in which bulk homogeneous nucleation occurred almost immediately at 25 °C and solutions prepared in this regime were not used in subsequent mineralization experiments. The clear region represents solution conditions in which no bulk precipitation occurred during the desired reaction time. Within these solution concentration parameters, heterogeneous nucleation of calcium phosphate mineral onto the derivatized substrates could be obtained.

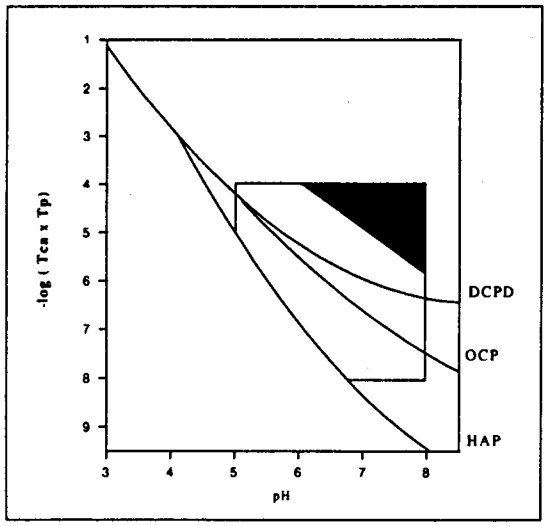

Figure 3. Solubility and solution chemistry regime for the mineralization experiments.

Scanning electron micrographs (SEM) of calcium phosphate mineralization (T_{Ca}=4.00 mM, T_{PO4}= 3.00 mM, pH 6.5)on the VMC substrates is shown in figures 4 and 5. In figure 4, the VMC substrate was removed from the mineralization solution prior to complete film formation. It can be seen that the crystals formed in the earlystages appear to be orientated perpendicular to

the substrate surface. After completion of coating formation, the surface is covered entirely by calcium phosphate (figure 5). X-ray diffraction of the sample showed the mineralized material to be pure octacalcium phosphate (OCP). SEM micrographs of the OCP coating, viewed on edge, show a densely formed and uniform film which is approximately 5 μm thick.

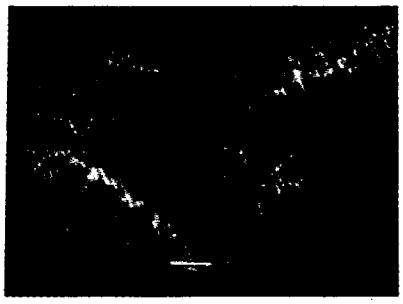

Figure 4
Early formation of OCP crystallites

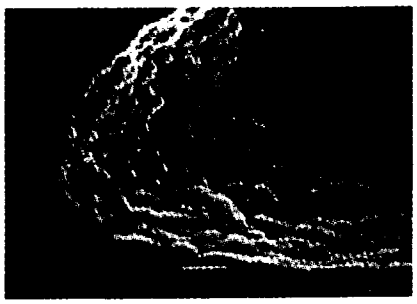

Figure 5
OCP Film on VMC

Conclusions and Future Directions

This preliminary work clearly demonstrates that the VMC and SIM calcium phosphate coatings provide an excellent means of producing bioactive and osteoinductive coatings. The VMC process produces porous structures that are interconnected and oriented. In addition the size and number of the pores can be controlled. The SIM process eliminates many of the shortcomings of plasma-spray deposition methods such as the presence of undesirable amorphous calcium phosphate phases. In addition, it is possible to coat microporous and complex-shaped materials without clogging the surface porosity. Biomechanical and histological evaluations have recently been completed and preliminary results indicate that the SIM process produces coatings which are stronger and have a faster rate of bone formation than coatings produced via a plasma sprayed route.

The aqueous deposition of bioactive minerals into porous substrates is truly unique to the fabrication of implant devices. While, this work is aimed at the formation of mineral coatings, the control of surface nucleation via surface modifications will also help in the development of strategies for inhibiting heterogeneous nucleation on surfaces. This could have tremendous impact in research areas, such as heart valve and pathological calcification, where mineral formation in vivo is undesired.

Acknowledgments

This work was supported by Battelle and the U.S. Department of Energy (Energy Research Office of Basic Energy Sciences and Office of Laboratory Technology Applications).

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Surface-induced mineralization: A new method for producing calcium phosphate coatings

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Calcium phosphate coatings were nucleated and grown from aqueous solution onto titanium metal substrates via surface-induced mineralization (SIM) processing techniques. This process is based on the observation that in nature organisms use biopolymers to produce ceramic composites, such as teeth, bones, and shells. The SIM process involves modification of a surface to introduce surface functionalization followed by immersion in aqueous supersaturated calcium phosphate solutions. This low-temperature process (< 100°C) has advantages over conventional methods of calcium phosphate deposition in that uniform coatings are produced onto complex-shaped and/or microporous samples. Additionally, because it is a low-temperature process, control of the phase and crystallinity of the deposited material can be maintained.

INTRODUCTION

The number of artificial hips implanted annually has risen to approximately 500,000 worldwide, and the use of dental implants is projected to approach 300,000/year in North America alone. These numbers reflect the increasing importance of developing methods to assure effective bone-to-implant fixation for load-bearing orthopedics.[1] It is known that clinical success requires the simultaneous achievement of a stable interface with connective tissue and a match of the mechanical behavior of the implant with the tissue to be replaced.[2] However, analysis of orthopedic implants over the past twenty years provides convincing evidence that failure originates at the implant–tissue interface.[3,4]

The high loads required of many implants restrict the selection of potentially useful materials. In addition to being biocompatible (or at least biotolerant), the implant material must possess adequate fracture and fatigue resistance. While metals or metal alloys meet many of the biomechanical requirements of implants, they have poor or nonexistent interfacial bonding between the metallic surface and the surrounding bone. In order to alleviate this problem, porous metal coatings have been applied to many implant surfaces. This facilitates bone ingrowth into the porous layer, thus improving fixation due to improved mechanical interlock. However, there remains concern about poor interfacial bonding between the porous metal coating and the surrounding bone and about the release of foreign elements into the body through implant corrosion and wear.

Many of the disadvantages of metallic implant devices can be diminished by the use of bioactive materials or coatings on the implant surface. Ducheyne[5] and Geesink[6] demonstrated that hydroxyapatite (HAP) surface coatings increased the rate of bone formation within a porous metal sample. Geesink further found that HAP-coated prosthetics could better bridge implant–bone interfacial gaps between cementless implants and existing bone. The ability of HAP coatings to bridge interfacial gaps increases the surgical tolerance limits, which could improve clinical success. Poor interfacial bonding between noncoated metal and bone also leads to the formation of a nonadherent, fibrous capsule in both soft and hard tissues,[2,5] which can result in movement at the implant–tissue interface and ultimate failure of the prosthetic device. However, HAP surfaces do not form fibrous tissues with existing bone, but rather an extremely thin, epitaxial bonding layer.[2]

Although there are many desirable features of bioactive surface coatings, an optimal technique for coating application has not yet been developed. Many methods have been explored, including dip coating/sintering, immersion coating, chemical vapor deposition (CVD),

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electrophoretic deposition, and sol-gel methods, with plasma spray-coating being the most popular.[2,7] Lacefield[7] found plasma coating to be the method of choice for forming a dense, adherent coating of HAP on metal substrates but cautioned that the coating of complex implant devices containing internal cavities was not feasible by this method. More serious was the formation in more than 50% of the coating attempts of an amorphous calcium phosphate material rather than stoichiometric HAP in the film.[8] In addition to concern about the bonding between the deposited coating and the underlying substrate, there also is concern over the long-term stability and quality of the HAP surface coatings produced using plasma spraying routes. And the ability of plasma spraying to coat within the pores of porous metal materials proves difficult because it is a line-of-sight process.

Surface-induced mineralization

The SIM method[9–12] offers one possible solution to current implant surface coating problems. This process is based on the observation that in nature organisms use various macromolecules to control the nucleation and growth of mineral phases.[13,14] These macromolecules usually contain functional groups, such as the sulfate groups in polysaccharides,[15] the carboxylic acids in aspartic and glutamic amino-acid-containing proteins,[16] and the phosphate groups of phosphoserine-containing proteins,[7] which are negatively charged at the crystallization pH.[18] It is speculated that these anionic groups act as chelators of ionic species present in the surrounding media, thus stimulating crystal nucleation. While nature's complex biomineralization process is far from being understood, the basic premise of surface functionalization to induce mineral deposition can be mimicked in the laboratory.

The SIM process "mimics" the idea of nature's template-mediated mineralization by chemically modifying substrates to produce a surface that induces heterogeneous nucleation from aqueous solutions. Rather than using complex proteins or biopolymers, the process employs surface modification schemes that place simple ionic functional groups on the underlying substrate. Using this approach, surface templates are formed that can induce the deposition of mineral phases from aqueous solutions. Derivatization schemes developed thus far include (1) chemical modification of plastics, (2) attachment of self-assembling monolayers, (3) electrochemical deposition of polymers, and (4) Langmuir-Blodgett (LB) film techniques.

Once the substrate has been chemically modified, it is placed into an aqueous solution containing soluble precursors of the desired ceramic material. Solution pH, ionic concentration, and temperature are maintained in a regime where the solutions are supersaturated with respect to the desired mineral phase, thereby creating the driving force for nucleation and growth. Because nucleation can be either homogeneous (in the bulk solution) or heterogeneous (on other solid surfaces), it is important to select solution parameters in which only heterogeneous nucleation on the modified substrate will occur. Thus a thorough understanding of the solution chemistry of the mineral system is required in order to select appropriate solution conditions.

The process is advantageous over conventional coating methods in that it is a low-temperature process that is adaptable to ceramic, polymeric, and metallic materials. Thus as other potential implant materials are introduced, the deposition of HAP onto these materials should be possible. Also, unwanted mineral phases can be avoided by careful selection of the mineralization solution conditions. Since it is not a line-of-sight process, uniform coatings on complex-shaped and microporous substrates is possible. And, finally, since no specialty equipment is needed, coating fabrication costs can be kept to a minimum.

MATERIALS AND METHODS

Surface derivatization

In this work, surface modification of titanium and titanium alloys was accomplished using self-assembled monolayers (SAMs) (Fig. 1).[9,19,20] The SAMs where attached to Ti-A16-V4 alloy wafers as follows: Prior to SAM formation, the wafers were cut to approximate sizes (1.25 × 2.5 cm), placed into Teflon racks, and ultrasonicated 2–3 min in chloroform (Aldrich) to remove any organic contaminates. Residual trace amounts of organic contamination then were removed by exposure to an air plasma for 10 min. Hydroxylation of the clean wafers was performed by treatment with 0.1*M* KOH solution for several min. The resulting hydroxides were protonated by immersing the wafers in 0.1*M* HNO3 solution for 10 min. Following the acid soak, the wafers were thoroughly washed with deionized water and blown dry with a stream of dry nitrogen gas.

SAM formation was accomplished by placing the wafers in a 1% silane:cyclohexane solution for 30 min. Following SAM formation, the wafers were rinsed in 2-propanol (Aldrich) in order to remove any residual silane. Finally, the wafers were sonicated in chloroform for 5 min to produce mirror-bright surfaces.

The terminus vinyl group of the alkylsilane tether subsequently was modified to sulfonic acid by exposure of the derivatized wafer to SO_3 gas in a reaction vessel for 1 min. Following sulfonation, the wafers

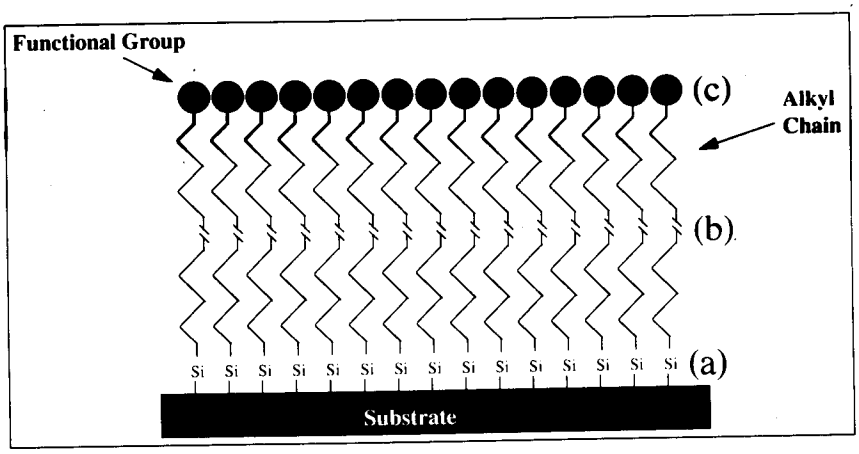

Figure 1. Self-assembled monolayer formation. The SAM molecule consists of three distinct parts: (a) a silane coupling agent that covalently attaches to the substrate surface; (b) the alkyl chain (from 3–33 methylene units); and (c) the functionalized end group used to induce mineral nucleation and growth (example includes $-COOH$, $-SO_3H$, $-PO_4H_2$, $-CH_3$, and $-NH_2$).

were removed, sonicated 10 min in deionized water, and blown dry with nitrogen gas.

The derivatized SAM surfaces on wafers were characterized at each step of the reaction sequence by X-ray photoelectron spectroscopy (XPS), contact angle measurements, and ellipsometry. XPS and contact wetting angle measurements confirmed the near-quantitative conversion (100%) of the vinyl SAM to the sulfonic acid.

In addition, both ^{13}C and ^{29}Si solid-state nuclear magnetic resonance (NMR) spectral studies (cross-polarization magic angle spinning, CP-MAS) were carried out on samples prepared on Ti powders. The ^{13}C spectrum indicated significant two-dimensional order by way of the signal intensity and the relatively narrow line width. In addition, utilization of the Bloch decay pulse sequence in similar silica-based monolayers indicated that complete, high-density monolayer formation had occurred.

Calcium phosphate mineralization

Solutions prepared using reagent-grade chemicals (Fisher Scientific) and deionized, reverse-osmosis (Millipore) CO_2-free water were filtered (0.22 μm Millipore filters) before use. The filters were prewashed in order to remove any residual wetting agents or surfacants. Calcium ion concentrations were determined by atomic adsorption or ion exchange using a cation-exchange column (Dowex) followed by the potentiometric titration of exchanged hydrogen with a standardized potassium hydroxide solution. Stock dihydrogen phosphate solution concentrations were determined potentiometrically by titration against standardized potassium hydrogen phthalate solutions.

Calcium phosphate deposition experiments were carried out in sealable glass containers. Supersaturated solutions were prepared by the slow addition of dihydrogen potassium phosphate to a calcium chloride solution. The pH was adjusted by the addition of 0.01*M* KOH solution. Following solution preparation, a rack containing the derivatized SAM surfaces was placed into the supersaturated solution, the vessel was sealed, maintained at room temperature, and gently stirred for the duration of the experiment. Following the desired reaction time, the substrates were removed from the mineralizing solution, rinsed with deionized water, and blown dry with N_2 gas. Samples then were analyzed by scanning electron microscopy (Electroscan), energy-dispersive X-ray analysis (Link Analytical), and X-ray diffraction (Phillips).

RESULTS

Surface derivatization

Because calcium phosphate minerals will not nucleate and grow on native titanium surfaces, surface modification of the substrate was necessary. In this work, modification of titanium and titanium alloys was accomplished using self-assembled monolayers (SAMs) (Fig. 1).[19,20] The alkyl silane of the general formula $Cl_3Si(CH_2)_nX$ (where X = a vinyl group) is adsorbed to the native surface oxide layer of a clean titanium substrate and "self-assembles" (an ordered aggregation of the silane molecules driven by the van der Waals interactions of the hydrocarbon chains). The trichlorosilane then undergoes covalent attachment to the surface via reaction with the surface hydroxyls. Once the silane is anchored to the surface, the remaining chlorosilane moieties undergo hydrolysis (as a result of the hydration layer of the titania), resulting in the formation of an anchored siloxane with two "dangling" hydroxyls. The "dangling" hydroxyls can undergo acid-catalyzed condensation with an adjacent

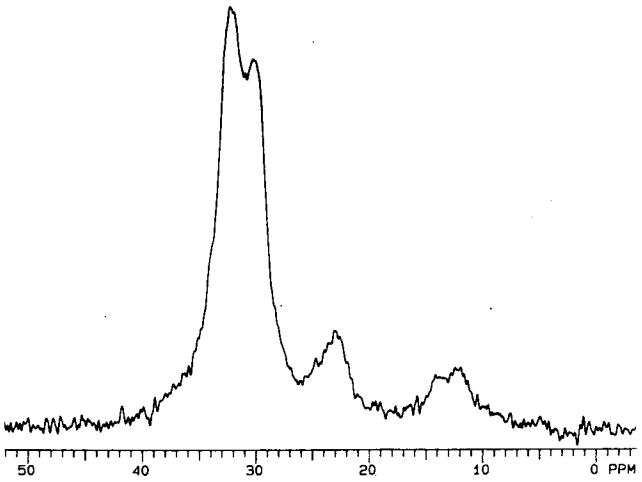

Figure 2. Solid-state CP-MAS ^{13}C NMR spectrum of an OTS monolayer on titania. The partially resolved peak at 13 ppm is due to the methyl terminus and the methylene bound to the silicon atom. The peak at 25 ppm is assigned to the methylene adjacent to the terminal methyl and the carbon β to the silicon. The internal methylenes compose the overlapping peak at 32 ppm. The spectral results are consistent with significant order and dense monolayer formation.

hydroxysiloxane to form the crosslinked structure traditionally associated with these SAMs (the attachment and hydrolysis of the trichlorosilane results in the formation of three equivalents of hydrogen chloride).

Verification of SAM attachment and structure was accomplished in experiments in which SAMs were formed on colloidal titania powder. Analysis of the ^{13}C spectrum of an octadecyltrichlorosilane (OTS) SAM on titania (Fig. 2) revealed several distinct carbon peaks.[21,22] At about 13 ppm are two poorly resolved peaks that have been assigned to the methyl terminus and the methylene α to the silicon atom. At approximately 25 ppm is a signal once again due to two different carbon atoms. This peak is assigned to the methylene adjacent to the methyl group and the methylene β to the silicon atom. The remaining internal methylene signals were lumped together in the peak at 32 ppm. The ^{29}Si spectrum verified that there was a covalent bond to the surface, as evidenced by a Ti-O-Si bond rather than simple physisorption to the surface. Analysis of this spectrum (Fig. 3) revealed that the primary component of the monolayer is the anchored silane with two "dangling" hydroxyls (found at −44 ppm), with a lesser component having one"dangling" hydroxyl and a single crosslinking siloxane bridge (found at about −55 ppm). Based on the precedent of the monolayers constructed on a silica surface, the anchored silane with two crosslinking siloxane bridges would be expected at about −62 ppm and was not observed for the monolayer built on titania.

Mineral deposition

The deposition of mineral phases from supersaturated aqueous solutions requires an understanding of the fundamentals of crystal nucleation and growth. In order for the formation of a mineral to occur, the solution must be supersaturated with respect to the precipitating mineral phase. For an inorganic, ionic salt, the crystallization driving force may be expressed as:

$$\Delta G = -RT \ln S \quad (1)$$

where ΔG is the Gibbs free energy of formation, R is the gas constant, T is the temperature, and S is the supersaturation. In the case of hydroxyapatite, the supersaturation may be written in terms of the lattice ion activities as:

$$S = (IP/K_{sp})^{1/9} \quad (2)$$

where $IP = (Ca^{2+})^5(PO_4^{3-})^3(OH)$ and K_{sp} is the solubility product (4.7×10^{-59} at 25°C). Ionic species activities

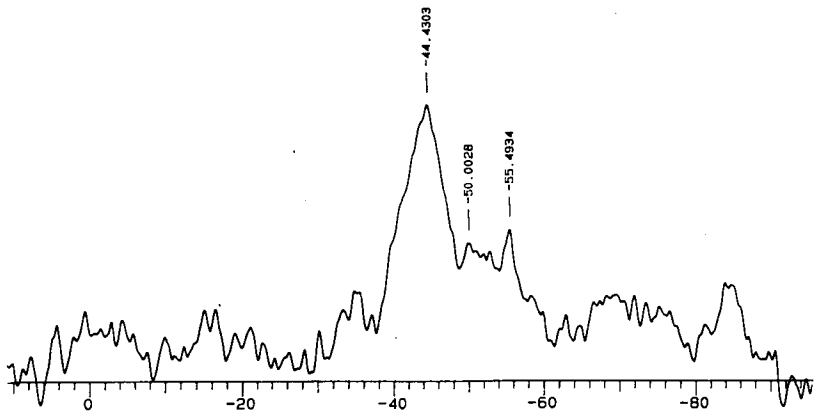

Figure 3. Solid-state CP-MAS ^{29}Si NMR spectrum of an OTS monolayer on titania. The peak at −44 ppm is due to the anchored alkylsilane with two dangling hydroxyl groups whereas the peak observed at −55 ppm corresponds to the anchored alkylsilane with a single dangling hydroxyl and one crosslinking siloxane bridge. Based on the precedent of silica-based systems, the anchored alkyl silane with two crosslinking siloxane bridges would be expected at −62 ppm.

TABLE I
Important Calcium Phosphate Minerals

Name	Formula	Ca/P Molar Ratio
Dicalcium phosphate dihydrate (DCPD)	$CaHPO_4 \cdot 2H_2O$	1.00
Octacalcium phosphate (OCP)	$Ca_8H_2(PO_4) \cdot 5H_2O$	1.33
Tricalcium phosphate (TCP)	$Ca_3(PO_4)_5$	1.50
Hydroxyapatite (HAP)	$Ca_5(PO_4)_3OH$	1.67

are calculated from the proton dissociation and ion pair-formation constants for calcium and phosphate, the mass balance, and electroneutrality conditions by successive approximations for the ionic strength.[23]

Table I lists the various calcium phosphate minerals and their chemical characteristics. Figure 4 shows the solubility isotherm for these phases as well as the range of solution conditions investigated for the mineralization of the derivatized substrate. As an initial selection, solution concentrations ranging from pH 5 to pH 8 and a $-\log (Ca \times PO_4)$ of 5 to 8 where chosen. The shaded region indicates solutions in which bulk homogeneous nucleation occurred almost immediately at 25°C, and solutions prepared in this regime were not used in subsequent mineralization experiments. The clear region represents solution conditions in which no

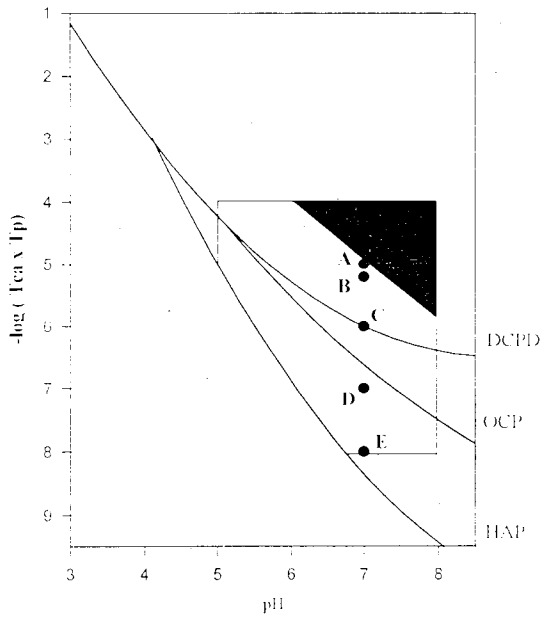

Figure 4. Solubility isotherms for various calcium phosphate phases. The boxed region represents a range of solution conditions investigated as potential mineralization solutions. The shaded area indicates solutions that are labile and where homogeneous nucleation occurs. DCPD = dicalcium phosphate dihydrate; OCP = octacalcium phosphate; and HAP = hydroxyapatite

bulk precipitation occurred during the desired reaction time. Within these solution concentration parameters, heterogeneous nucleation of calcium phosphate mineral onto the derivatized substrates could be obtained.

Within the heterogeneous nucleation regime, several specific solution conditions were investigated in order to determine (a) the time required for film formation to occur, and (b) any phase preference of the deposited material. Solutions A and B had the highest driving force for HAP crystallization ($S_{(HAP)}$ = 30.1 and 25.7, respectively) and were supersaturated with respect to all of the calcium phosphate phases. Solution C ($S_{(HAP)}$ = 12.3) was undersaturated with respect to dicalcium phosphate dihydrate (DCPD) but supersaturated with respect to the other phases. Solutions D and E were undersaturated for all phases except HAP ($S_{(HAP)}$ = 4.59 and 1.08, respectively). Although most of the solutions were supersaturated with respect to several calcium phosphate phases, the solution calcium to phosphate molar ratio for all solutions was held at 1.67, corresponding to HAP stoichiometry.

All solutions studied produced mineral formation on the SAM-modified Ti substrates. However, the rate of mineral deposition of the various solutions varied widely. It is important to note that in all cases the coatings were deposited before any particles were observed in the bulk solutions. When the supersaturation was high, the solution tendency toward homogeneous nucleation also was high. Therefore care was taken to observe these solutions frequently and to remove the substrates at the first observable sign of any precipitate in the bulk solution. Solutions A and B, having the highest driving force for mineralization, underwent homogeneous precipitation within several hours. During this time, calcium phosphate mineral was deposited on the substrate, but the coating was very thin and discontinuous. These solutions, while metastable for several hours, spontaneously precipitated before complete nucleation and growth could occur on the substrate surface. Solutions C and D produced nearly continuous (15–10 μm) calcium phosphate films within 24–48 h. Because solution E was only slightly supersaturated with respect to HAP, the time required for nucleation and growth to a 1-μm coating was on the order of days.

The calcium phosphate coatings were analyzed by scanning electron microscopy (SEM) and energy dispersive X-ray analysis (EDS). Figure 5 shows a SEM micrograph of the coating resulting from solution D, which produced a coating of small particles that are difficult to resolve. The chemical composition of the coating, as analyzed by EDS, showed a calcium to phosphorus atomic ratio of approximately 1.7, which is in close agreement with the atomic ratio of 1.67 for HAP, and X-ray diffraction confirmed the presence of HAP. Comparatively, a SEM micrograph of a coating mineralized from solution C (Fig. 6) shows a different

Figure 5. Scanning electron micrograph of a calcium phosphate coating mineralized from solution D (Fig. 4). Crystallites are quite small and difficult to discern. Ca/P atomic ratio as measured by EDS was 1.7.

morphology than the coating produced from solution D. In this case, the precipitated film had a very distinct rosette-like morphology typical of octacalcium phosphate crystals. EDS analysis of this material showed a calcium to phosphorus atomic ratio of 1.4, which is similar to that of 1.33 for OCP. X-ray powder diffraction results also confirmed the presence of OCP as the dominant phase. The formation of an OCP coating from solution C was unexpected since HAP is the least soluble phase and the solution was only slightly supersaturated with respect to OCP. In addition, the solution calcium to phosphorus molar ratio was set for HAP stoichiometry. Since the coating from solution C had a Ca/P ratio of 1.4, it is possible that the coating was a mixture of OCP and HAP phases. This was probably the result of an initial coating of OCP undergoing phase transformation to HAP via dissolution and reprecipitation mechanisms. The OCP–HAP phase transformation mechanism is an area that we currently are exploring in greater detail.

Figure 6. Scanning electron micrograph of a calcium phosphate coating nucleated from solution B (Fig. 4). The morphology shown is typical of OCP crystals, and the Ca/P atomic ratio was 1.4.

Following the demonstration of the surface-induced deposition concept using smooth, flat Ti substrates, attempts were made to apply calcium phosphate coatings onto high-surface area beaded Ti samples. Figure 7 shows a high-surface area beaded Ti-6Al-4V alloy rod used for preliminary biomechanical and histological tests. This type of sample illustrates that the SIM-produced coatings could deposit onto a complex surface geometry without reducing or eliminating the high porosity or surface area of the original material. Thus the mechanical interlocking capabilities of porous implants can be maintained with the addition of a calcium phosphate coating.

Scanning electron micrographs show that unlike a plasma-sprayed technique (Fig. 8) in which the high surface area and topography of the substrate were greatly altered by a thick calcium phosphate layer, the SIM coatings did not noticeably change the surface structure (Fig. 7 insert).

CONCLUSIONS AND FUTURE DIRECTIONS

This preliminary work clearly demonstrates that SIM calcium phosphate coating provides an excellent

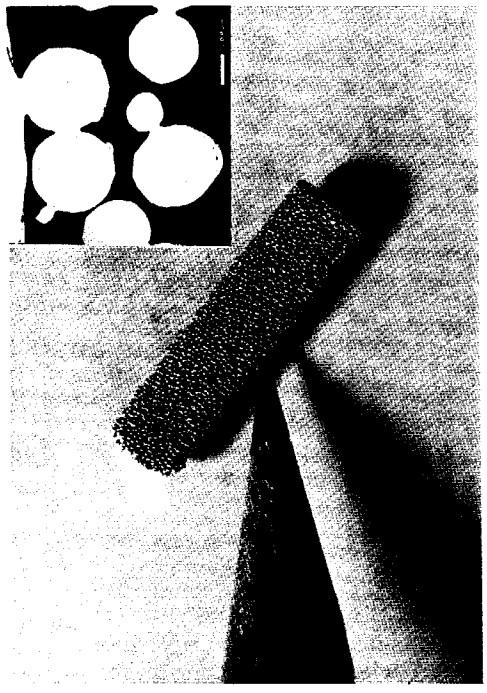

Figure 7. Porous metal coating on a Ti-6Al-4V alloy rod. The beaded surface provides cavities for bone ingrowth. Insert: Optical micrograph of a SIM-coated implant cut in cross-section.

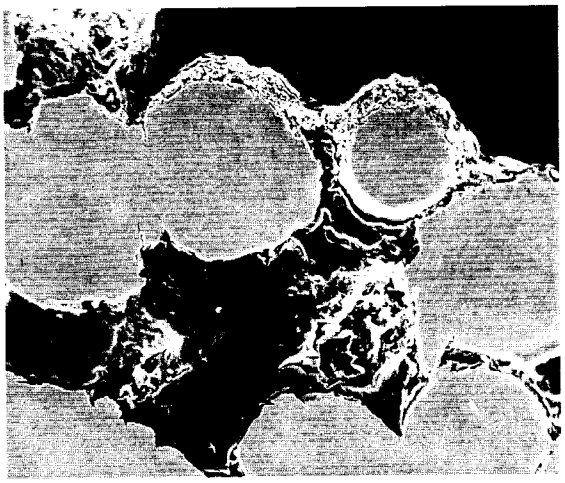

Figure 8. Scanning electron micrographs of plasma-sprayed porous-rod cross-section. The surface porosity is clearly clogged by the plasma-sprayed calcium phosphate coating, thereby reducing or eliminating the ability of new bone formation within the implant cavities.

means of producing bioactive and osteoinductive coatings. The process eliminates many of the shortcomings of plasma-spray deposition methods, such as the presence of undesirable amorphous calcium phosphate phases. In addition, it is possible to coat microporous and complex-shaped materials without clogging the surface porosity. Biomechanical and histological evaluations recently have been completed, and preliminary results indicate that the SIM process produces coatings that are stronger and have a faster rate of bone formation than coatings produced via a plasma-sprayed route. An in-depth analysis of the animal results will be forthcoming in a subsequent paper.

The aqueous deposition of bioactive minerals onto modified substrates is truly unique to the fabrication of implant devices. Just as nature uses a complex scheme to mineralize bone, teeth, and shells, SIM offers a process for forming a variety of mineral/substrate combinations. While this work is aimed at the formation of mineral coatings, the control of surface nucleation via surface modifications also will help in the development of strategies for inhibiting heterogeneous nucleation on surfaces. This could have a tremendous impact in research areas such as heart valve and pathological calcification, where mineral formation *in vivo* is not desired.

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Technical Note

Histological and biomechanical evaluation of calcium phosphate coatings applied through surface-induced mineralization to porous titanium implants

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The purpose of this pilot study was to evaluate surface-induced mineralization (SIM) as a potential technique to apply ceramic coatings to metal orthopaedic implants. Cylindrical titanium porous-coated implants were either coated by SIM or plasma-spray (PLS) techniques with calcium phosphate, or left uncoated (CTL). The implants were bilaterally implanted into the intramedullary canal of the proximal femur of 24 adult New Zealand white rabbits segregated into the following groups: PLS/CTL, SIM/CTL, and SIM/PLS. After 6 weeks *in vivo*, biomechanical and histologic evaluations were completed. Biomechanically, SIM had consistently greater mechanical interlock than PLS implants. However, CTL implants had greater mechanical interlock than both PLS and SIM. The small sample size prevented statistical evaluation and definitive biomechanical conclusions. Histologically, SIM and PLS had significantly greater ingrowth than CTL implants ($p < 0.05$). The SIM coating technique produced similar ingrowth characteristics as standard PLS coatings, yet may prevent osteolysis by providing a stronger, more reliable, covalent bond between the ceramic and metal.

INTRODUCTION

The use of porous metal implants for uncemented use in orthopedic total joint replacements has grown in popularity in recent years. The success of these uncemented implant systems depends on the ability of bone to grow into the metal pores of the implant providing mechanical interlock.

Augmenting implant stability by acelerating bone ingrowth with ceramic materials has been the focus of numerous investigations.[1–5] Plasma-spray hydroxyapatite (HA) and calcium phosphate (CP) are the most popular ceramic implant coatings and have been shown to enhance bone growth along their surface[2,4,5] and support considerable interfacial shear stress.[2,5] However, human implant retrieval studies of plasma-spray HA and CP-coated titanium alloy implants reveal evidence of loose HA and CP particles with accompanying osteolysis.[3,6–8] Ceramic particulates have been demonstrated to inhibit bone cell proliferation and increase cytokine production *in vitro*.[9,10] The extensive bone formation surrounding plasma-sprayed, ceramic-coated implants may be a considerable advantage for bony anchorage; however, the bonding strength between HA and the titanium substrate is a limiting factor for implant fixation.[11] Given the adverse tissue reactions of ceramics, the long-term efficacy of plasma-spray, ceramic-coated implants is uncertain. The use of surface-induced mineralization (SIM), which chemically bonds the ceramic to the metal surface, offers many advantages over current plasma-spray technology.[12] SIM provides a covalent chemical bond between the metal and ceramic which could potentially prevent shedding of particulate CP while providing a stable stoichiometric ceramic film on the surface of the metal. In addition, the stoichiometric SIM CP film is thought to be chemically superior to the amorphous plasma-spray HA film and may prove to be a more effective stimulate for bone induction.

OBJECTIVE

The objective of this pilot study was to evaluate the *in vivo* biological response to SIM CP coated metal

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implants compared to plasma spray-coated and uncoated implants using a rabbit model.

MATERIALS AND METHODS

Prostheses

Forty-eight 18.5-mm-long, porous-coated, Ti-6AL-4V cylindrical implants with a core diameter of 3 mm were sintered with titanium beads with an average porosity of 400 μm. The plasma HA coatings ($Ca_{10}[PO_4]_6[OH]_2$) were applied using standard techniques[13] at an average thickness of 100 μm. The SIM CP implants had an average ceramic coating thickness of 10 μm using the application technique described below.

SIM coating technique

Calcium phosphate coatings were nucleated and grown from an aqueous solution onto titanium metal substrated through SIM techniques.[12] The SIM process involves modification of a surface to introduce surface functionalization followed by immersion in aqueous supersaturated calcium phosphate solutions (Fig. 1). This low-temperature process (25°C) provides precise control of the phase and crystallinity of the deposited material allowing uniform coating to be applied to complex shapes and microporous structures.

Animals and surgery

Twenty-four adult, 5-kg New Zealand white rabbits were systematically assigned to receive bilaterial implants and categorized into the following groups: plasma-spray HA (PLS) and uncoated (CTL) (n = 8); SIM and CTL (n = 8); and PLS and SIM (n = 8). NIH guidelines for the care and use of laboratory animals (NIH Publication no. 85-23, Rev. 1985) were strictly observed.

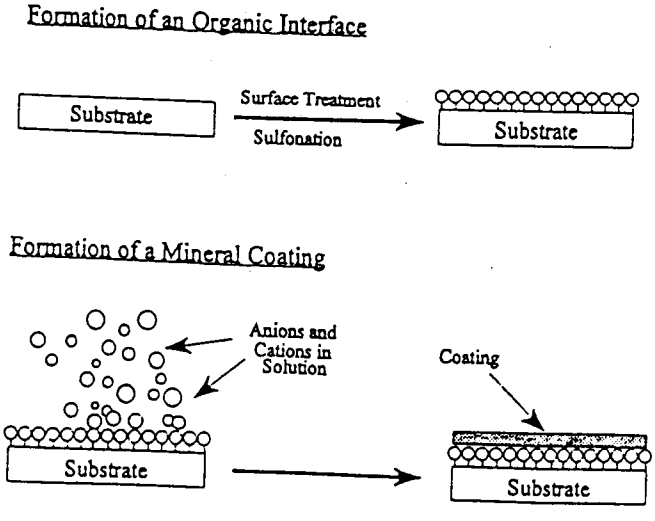

Figure 1. Diagram of SIM technique.

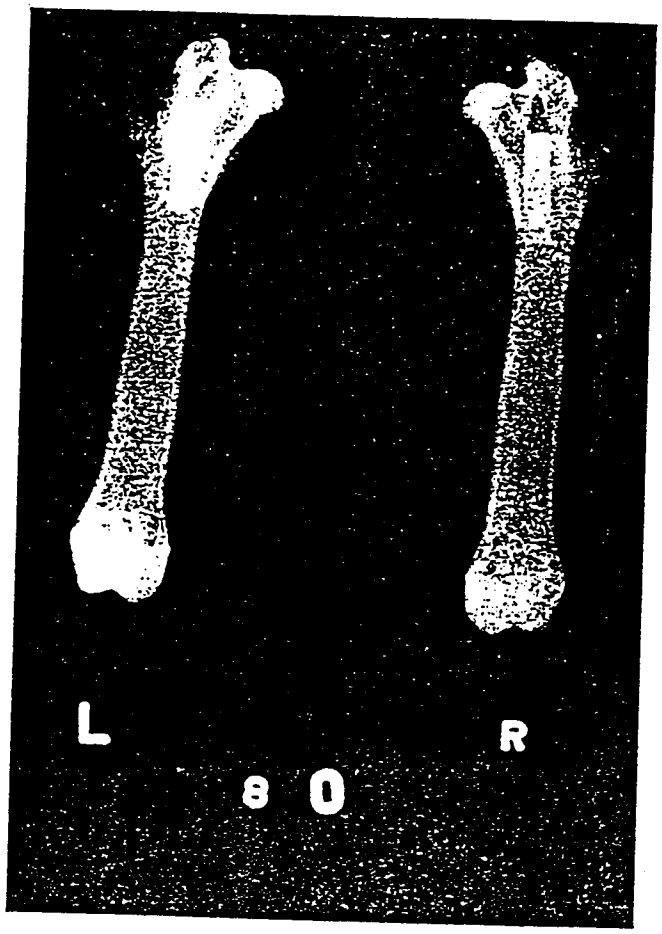

Figure 2. *Ex vivo* radiograph of implants in proximal femurs.

All surgical procedures were completed under sterile conditions. Animals were anesthesized with intramuscular injections of 5 mL of ketamine (100 mg/cc), 2.5 mL of Rompun (20 mg/cc), and 1 mL of acepromazine (10 mg/cc) at a dose of 1 mL/kg. A skin incision was made over the greater trochanter and the trochanteric fossa was exposed. From this approach, the intramedullary canal was gently reamed in a progressive stepwise fashion to allow insertion of the implant. All implants were inserted with a tight fit until the proximal aspect of the implant was flush with the surrounding bone (Fig. 2). Incisions were sutured closed in layers and the procedure was repeated on the contralateral limb. The animals received buprenorphine (0.02–0.05 mg/kg) analgesic for pain as needed and were allowed to move freely in their cages after surgery. Animals were sacrificed 6 weeks after surgery with an overdose of sodium pentobarbital and their

femurs harvested for biomechanical and histologic assessments.

Biomechanical testing

Biomechanical tests were performed on two animals from each group. Specimens were stored frozen at −20°C and thawed to room temperature prior to testing. Specimens were wrapped in saline-soaked gauze to assure proper hydration prior to and during testing. Radiographs were taken of femoral specimens to provide a guideline for cortical transection (Fig. 2). The femurs were cut transcortically 2 mm distal and proximal to the implant. The bone implant was placed on a self-aligning fixture which circumferentially supported the surrounding cortical bone while allowing translation of the central implant. A servohydraulic materials testing machine (Instron, Canton, MA) was used to apply an axial load at a constant loading rate of 2 mm/min until failure. Force displacement curves were outputted and collected using an X-Y recorder (Hewlett Packard, Dallas, TX). The external surface area of the porous coating (290 mm^2) was used to calculate interfacial shear strength.

Histologic assessment

Histologic analyses were performed on six animals from each group. Radiographs were taken of the femoral specimens after sacrifice (Fig. 2). The femurs were cut transcortically distal and proximal to the implant. The tissue was fixed in alcohol formalin and embedded in methyl methacrylate using standard methods.[14] Serial cross sections (200 μm) were taken through the bone–implant composite using a low-speed bone saw (Buehler, Lake Bluff, IL) and later ground to <50 μm using a precision microgrinder (Exakt, Oklahoma City, OK) Six histologic sections were analyzed histomorphometrically from each specimen, two sections each from the proximal, middle and distal aspect of the implant. Sections were stained with a light green bone stain countered with basic fuchsin. Histomorphometric analyses were performed using a Leica DMRB microscope (Leica, Deerfield, IL), Sony CCD color video camera (San Jose, CA), and Optimas image analysis software (Seattle, WA). The percentage of the available pore area of the implant that contained ingrown bone was quantified through automatic color differentiation of the image analysis software.

Statistical analysis

No statistics could be run on the mechanical tests owing to the small sample size. The histologic results were analyzed using an analysis of variance followed by Duncan's multiple comparison procedure. The level of significance for all statistical tests was set at $p = 0.05$ and the statistical power for the study design was $\beta = 0.80$.

RESULTS

The pushout tests showed that the SIM implants had consistently greater mechanical interlock than the PLS implants. However, the CTL implants had greater mechanical interlock than both the PLS and SIM (Fig. 3).

The histologic results indicated that the SIM and PLS groups had significantly greater ingrowth than the CTL group ($p < 0.05$), but SIM and PLS were not significantly different. The histologic results are summarized in Figure 4.

DISCUSSION AND CONCLUSION

Previous studies have documented enhanced and accelerated osteointegration into porous metal implants with the application of plasma-spray hydroxyapatite and tricalcium phosphate coatings.[2,4,15] The present study demonstrated that surface-induced mineralization is a viable alternative to plasma-sprayed ceramic coatings. The bone ingrowth and pushout strength of SIM coated implants were equivalent to those implants coated using plasma-spray techniques. Plasma-spray techniques have been associated with the presence of ceramic particulate debris in the soft tissues surrounding the implant which can lead to adverse tissue reactions, osteolysis, and eventual loos-

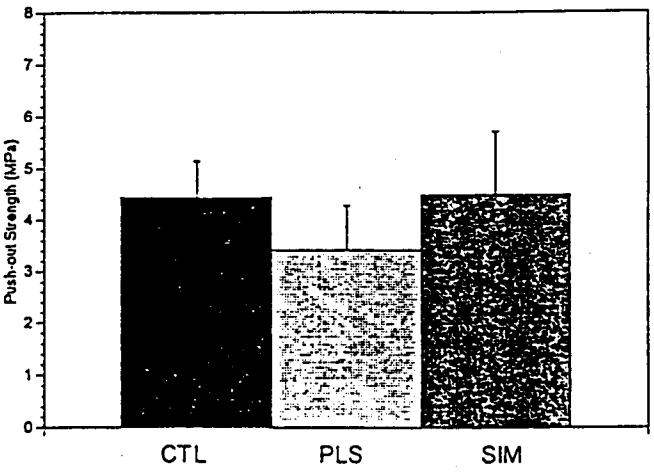

Figure 3. Biomechanical results from pushout tests.

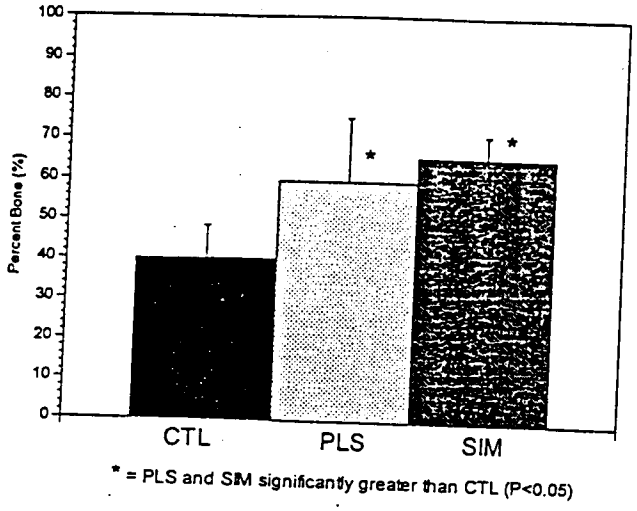

Figure 4. Histologic results of percent bone within the metal pores.

ening and implant fixation failure.[3–6,10] Alternative coating methods which provide greater ceramic–metal shear strength and fatigue resistance would prevent shedding of the coating *in vivo* and obviate adverse tissue reactions. Unpublished experiments in our lab indicate that SIM techniques secure the ceramic coating to the metal through strong covalent chemical bonds which are more resistant to shear and cyclic loading than plasma-spray techniques. In addition, uniform stoichiometric ceramic coatings can be applied using SIM techniques without "shadowing," which is prevalent in plasma-spray techniques.

The histologic results of the present study indicate that SIM and plasma-spray coatings provided similar ingrowth characteristics. The majority of SIM and PLS ceramic-coated implants exhibited a thin ring of bone on the surface of the coated metal, even at levels close to the implant core. This pattern contrasts that of the uncoated implants where bone was present only at the periphery. The hydroxyapatite and calcium phosphate coatings may provide an environment conducive to cell anchorage and adhesion, leading to enhanced and accelerated bone ingrowth into the metal pores.[2,16]

This animal model employed unloaded implants allowing ingrowth to occur without implant micromotion. A more appropriate model would involve a loaded implant, incorporating the variable of micromotion.[17,18] Micromotion would induce shear loads on the implant coating, possibly producing wear debris and adverse affects on ingrowth. Future studies will evaluate loaded SIM ceramic-coated implants to evaluate wear debris and bone ingrowth under simulated physiologic loading conditions.

Mechanical testing confirmed that both PLS and SIM techniques provided stable osteointegration of the implants within the proximal femoral cancellous bed with similar pushout strengths as those found by others using other models.[19] However, the uncoated implants provided stronger mechanical interlock than the ceramic-coated implants. These biomechanical results tend to conflict with the histologic results. Others have found greater shear strength for uncoated implants than for ceramic-coated implants, although histologically greater bone ingrowth was found in ceramic-coated implants.[20–22] Most studies have confirmed greater bone ingrowth and shear strength for HA-coated implants, especially at early time points.[18,23,24] It should be noted that definitive conclusions on the mechanical competency of the ceramic coated implants cannot be drawn from this investigation owing to the small sample size.

In summary, calcium phosphate coatings deposited through surface-induced mineralization on cylindrical porous metal implants induced similar bone ingrowth characteristics as plasma-spray hydroxyapatite coatings. The bone ingrowth within the metal pores of ceramic-coated porous implants, both SIM and PLS, were superior to that found in uncoated porous implants. This increased bone formation may result from enhanced attachment of bone-forming cells to the ceramic surface. Additional studies are required to document the short- and long-term bone ingrowth characteristics and mechanical competency of SIM ceramic-coated implant under loaded conditions.

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[45] Date of Patent: Sep. 28, 1999

[54] **THIN FILM COMPOSITION WITH BIOLOGICAL SUBSTANCE AND METHOD OF MAKING**

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[22] Filed: **Feb. 20, 1998**

[51] **Int. Cl.**[6] **A61K 9/00**; A61F 13/00; A61F 2/00

[52] **U.S. Cl.** **424/400**; 424/422; 424/423; 424/426

[58] **Field of Search** 424/400, 422, 424/423, 426; 623/16; 523/113, 114, 115; 428/426, 457

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Attorney, Agent, or Firm—Paul W. Zimmerman

[57] **ABSTRACT**

The invention provides a thin-film composition comprising an underlying substrate of a first material including a plurality of attachment sites; a plurality of functional groups chemically attached to the attachment sites of the underlying substrate; and a thin film of a second material deposited onto the attachment sites of the underlying substrate, and a biologically active substance deposited with the thin-film. Preferably the functional groups are attached to a self assembling monolayer attached to the underlying substrate. Preferred functional groups attached to the underlying substrate are chosen from the group consisting of carboxylates, sulfonates, phosphates, optionally substituted, linear or cyclo, alkyl, alkene, alkyne, aryl, alkylaryl, amine, hydroxyl, thiol, silyl, phosphoryl, cyano, metallocenyl, carbonyl, and polyphosphate. Preferred materials for the underlying substrate are selected from the group consisting of a metal, a metal alloy, a plastic, a polymer, a proteic film, a membrane, a glass or a ceramic. The second material is selected from the group consisting of inorganic crystalline structures, inorganic amorphus structures, organic crystalline structures, and organic amorphus structures. Preferred second materials are phosphates, especially calcium phosphates and most particularly calcium apatite. The biologically active molecule is a protein, peptide, DNA segment, RNA segment, nucleotide, polynucleotide, nucleoside, antibiotic, antimicrobal, radioisotope, chelated radioisotope, chelated metal, metal salt, anti-inflamatory, steriod, nonsteriod anti-inflammatory, analgesic, antihistamine, receptor binding agent, or chemotherapeutic agent, or other biologically active material. Preferably the biologically active molecule is an osteogenic factor the compositions listed above.

25 Claims, No Drawings

THIN FILM COMPOSITION WITH BIOLOGICAL SUBSTANCE AND METHOD OF MAKING

This invention was made with Government support under Contract DE-AC06 76RLO 1830 awarded by the U.S. Department of Energy. The Government has certain rights in the invention.

FIELD OF THE INVENTION

This invention provides a thin-film coating incorporating biologically active materials.

BACKGROUND OF THE INVENTION

A method for depositing thin film layers on to surfaces modified with organic functional groups and products formed thereby is disclosed in published PCT application WO 91/17286. Refinements of the method are disclosed in Bunker, et als. *Ceramic Thin Film Formation on Functionalized Interfaces Through Biomemetic Processing*, Science, 264, 48–55 (1994) and Rieke, et als. "*Biomemetic Thin-Film Synthesis*", in *Supramolecular Architecture: Synthetic Control in Thin-Films and Solids*, T Bein ed., American Chemical Society, Washington D.C., 61–75, (1992), and in "Bioactive Void Metal Compositites For Orthopedic Implant Devices," Campbell, et als., National Research Society Proceedings, 414, 177, (1996).

In the process for providing a film product, an underlying substrate of a first material is chemically modified on at least one surface by attaching functional groups which provide nucleation sites for inducing crystallite growth of an inorganic second material on the underlying substrate, and contacting at least one chemically modified surface with a liquid solution of precursors of the inorganic second material for a sufficient period of time for the crystallite growth of a second material formed from the precursors of the inorganic second material in the liquid solution onto the modified underlying substrate by nucleation of the second material on the nucleation sites thereby forming inorganic crystallite second material growth onto the nucleation sites, the nucleation sites being chemically attached to the underlying substrate. Materials that mimic biological materials such as porous metal composites coated with hydroxyapatite have been produced by this process.

Processes for delivery of biologically active molecules such as bone morphogenic proteins have also been previously disclosed. See for example U.S. Pat. No. 5,385,887 and references cited therein. However the incorporation of biologically active materials, such as proteins into thin-film coatings has not previously been demonstrated. Release of such incorporated materials has not previously been suggested.

SUMMARY OF THE INVENTION

The invention provides thin-film composition comprising an underlying substrate of a first material including a plurality of attachment sites; a plurality of functional groups chemically attached to the attachment sites of the underlying substrate; and a thin film of a second material deposited onto the attachment sites of the functional groups, and a biologically active substance deposited with the thin-film. Preferably a self assembling monolayer containing functional groups is attached to the underlying substrate. Preferred functional groups attached to the underlying substrate are chosen from the group consisting of carboxylates, sulfonates, optionally substituted, linear or cyclo, alkyl, alkene, alkyne, aryl, alkylaryl, amine, hydroxyl, thiol, silyl, phosphoryl, cyano, metallocenyl, carbonyl, phosphates and polyphosphates. Preferred materials for the underlying substrate are selected from the group consisting of a metal, a metal alloy, a plastic, a polymer, a protein film, a membrane, a glass or a ceramic. The second material is selected from the group consisting of inorganic crystalline structures, inorganic amorphus structures, organic crystalline structures, and organic amorphus structures. Preferred second materials are phosphates, especially calcium phosphates and most particularly octacalcium phosphates ("OCP"). Especially preferred are componds selected from the group consisting of octacalcium phosphate, hydroxyapatite and carbonate apatite.

The biologically active molecule is a protein, peptide, DNA segment, RNA segment, nucleotide, polynucleotide, nucleoside, antibiotic, antimicrobal, radioisotope, chelated radioisotope, chelated metal, metal salt, anti-inflammatory, steroid, nonsteroid anti-inflammatory, analgesic, antihistamine, receptor binding agent, or chemotherapeutic agent, or other biologically active material. Preferably the biologically active molecule is an osteogenic factor. In an alternative embodiment the invention may be described as a method for preparing a multi component coating which comprises treating a first material to produce a plurality of attachment sites on its surface, attaching a plurality of functional groups to the attachment sites, incorporating the functional groups into a thin-film of a second material deposited onto the material and providing a biologically active material which is incorporated into the thin film deposited onto the material. The method is suitable for providing all the compositions listed above.

In alternative embodiments the invention provides a bone fixation device and a method for delivery of drugs or therapeutic agents.

DETAILED DESCRIPTION OF THE INVENTION

While the prior art has provided thin-film deposition methods, and compositions for drug delivery or sustained release, the art has not provided a method for depositing thin-films incorporating biologically active substances. The present work demonstrates that useful thin-films incorporating biologically active substances can be deposited by a variation of the method disclosed in published PCT application WO 91/17286. Small molecules, such as antibiotic and anti-cancer drugs, and proteins such as bovine serum albumin and transforming growth factor β1 have been successfully incorporated into calcium phosphate coatings on titanium and titanium alloy surfaces modified using self assembling monolayers. The preparation of these examples is set out in detail below.

EXAMPLE 1

Surface Functionalization Using Self Assembling Monolayers (SAM)

Prior to SAM formation, titanium or titanium alloy wafers were cut and polished on one side. The wafers were placed on racks and washed with acetone and ethanol. After being sonicated in chloroform for 2–3 minutes, the wafers were exposed to an air plasma for 20 minutes to remove organic residues. The wafers were treated with 0.1M KOH for 2 minutes. The wafers were then immersed in 0.1M HNO_3 for 10 minutes. The wafers were blown dry after a thorough washing with deionized water. The wafers were further dried in a nitrogen stream for 2 hours. The self assembling

monolayers were formed by treating the wafers in a 0.5 wt % terminal alkenyl-silane such as 1-(trichlorosilyl)-undec-10-ene in cyclohexane solution for 30 minutes. The wafers were rinsed in 2-propanol, sonicated in chloroform for 5 minutes and blown dry with nitrogen. The terminal vinyl group of the alkenyl silane was converted to a sulfonic acid by exposure to SO_3 gas for 1 minute. Following sulfonation the wafers were sonicated in deionized water for 10 minutes and blown dry with nitrogen.

EXAMPLE 2

Calcium Phosphate Film Deposition

A supersaturated calcium phosphate solution containing 5 mM $CaCl_2$, 1.5 mM KH_2PO_4 and 1.5 mM Na_2HPO_4 was prepared by mixing 1.5 ml of 0.1M KH_2PO_4 and 1.5 ml of 0.1M Na_2HPO_4 stock solutions into 92 ml of deionized water, followed by the slow addition of 5.0 ml 0.1M $CaCl_2$ solution. The combined solution was stirred for 3 minutes and derivatized wafers prepared as in Example 1 above, were immersed in the solution and taken out just before the solution precipitated (about one hour). The wafers were then rinsed with deionized water and blown dry with nitrogen. The process may be repeated several times to achieve a desired thickness. Films up to 20 microns were obtained in this way. The resulting calcium phosphate films were characterized by X-ray diffraction and scanning electron microscopy. The thin film prepared as described showed a single phase of octacalcium phosphate.

EXAMPLE 3

Incorporation and Release of Protein and Drug Molecules by Calcium Phosphate Films

Stock solutions of 5.6 mg/ml of 5 fluro-uracil (5-FU), 1 mg/ml tetracycline, 0.65 mg/ml bovine serum albumin (BSA) and 0.33 μg/ml transforming growth factor -β1 (TGF-β1) were prepared and adjusted to pH 6.5 before use. After a calcium phosphate deposition cycle as described in Example 2, wafers were immersed in the desired stock solution for 1 hour, rinsed and blown dry (one adsorption cycle). If desired additional calcium phosphate deposition as described in Example 2 may be carried out followed by additional adsorption from stock solution. Stock solution concentrations were determined before and after each adsorption cycle and the amount adsorbed was determined by the deference. Concentrations of incorporated sample and released materials are tabulated below. Concentrations of 5-FU were determined by direct measurement of the adsorption at 266 nm. BSA was determined by the bicinchonic acid method. P. K. Smith, et al., "Measurement of Protein using Bicinchonic Acid," Analytical Biochemistry, 150, 76–85 (1985), and by adsorption at 562 nm. Concentration of TGF-β1 was determined by the Quantikine immunoassay, (R&D Systems). Tetracycline concentrations were determined by adsorption at 275 nm.

TABLE 1

TGF-β1 absorbed into calcium phosphate film as a function of absorption cycles

Adsorption Cycles	TGF-β1 Absorpbed (μ/cm²)
0	0
1	0.00686
2	0.260
3	0.374
4	0.400
5	0.404
6	0.407

Release of Incorporated Proteins and Drugs

Wafer samples processed several times as described above were placed in 0.1M NaCl (saline) solution at 25° C. Wafers were periodically transferred to fresh saline and the concentration in the old saline was measured directly as in Example 3 by evaporating to dryness then analyzing. Table 2 shows release of 5-FU and Table 3 shows release of BSA.

TABLE 2

5-FU release in 0.15M NaCl at 25° C. from a 5 Micron Thick OCP Film

Time (hours)	5-FU Released (μ/cm²)
0	0
0.133	0.004
0.250	0.008
0.367	0.012
6.00	0.193
22.00	0.707
27.50	0.884
46.50	1.495
73.00	2.347
77.00	2.476
143.00	4.598
167.00	5.370

EXAMPLE 4

Direct Incorporation

The procedure of Example 2 is repeated and a selected drug such as tetracycline or 5 fluorouracil, a DNA segment, or a protein such as TGF-β1 is slowly added with the $CaCl_2$ by titration. In this manner the adsorption and deposition occur at the same time. This procedure can be used with any biologically active molecule that can be dissolved or suspended in the $CaCl_2$ solution. Insoluble or unstable molecules may be adsorbed as in Example 3.

TABLE 3

BSA Release from OCP films in 0.15M NaCl at 25° C.

Time (hour)	BSA Released (mg/m²)
0	0
0.25	0.0014
0.67	0.0011
1.67	0.0015
2.67	0.0019
4.67	0.0024
20.00	0.0042
28.00	0.0042
44.00	0.0065
167.00	0.0062

Any biologically active molecule may be deposited into the film using the technique of either example 3 or example 4. The method can be used for example to incorporate molecules of any of the following examples onto or into the film layer: a protein, peptide, DNA segment, RNA segment, nucleotide, polynucleotide, nucleoside, antibiotic, antimicrobial, radioisotope, chelated radioisotope, chelated metal, metal salt, anti-inflamatory, steriod, nonsteriod anti-inflammatory, analgesic, antihistamine, receptor binding agent, or chemotherapeutic agent. The film may be any organic or inorganic film that can be deposited on a functionalized surface. The surface may be any material having attachment sites for organic functional groups. The attachment sites may be generated or may be natural to the material. Any functional group that will support deposition of a second material may be used. The preferred materials are biologically compatible alloys suitable for implantation into humans, particularly porous metal implants of titanium or titanium alloys.

DEFINITIONS

"Biologically active substance means a substance that produces a detectable result other than a foreign body response when placed in contact with a living organism.

We claim:

1. In a thin-film composition comprising an underlying substrate of a first material including a plurality of attachment sites; a plurality of functional groups chemically attached to the attachment sites of the underlying substrate; and a thin film of a second material deposited onto the attachment sites of the underlying substrate, the improvement comprising a biologically active substance incorporated with the thin film of the second material.

2. A composition according to claim 1 wherein a self assembling monolayer containing functional groups are attached to the underlying substrate.

3. A composition according to claim 1 wherein the functional group attached to the underlying substrate is chosen from the group consisting of carboxylates, sulfonates, phosphates, optionally substituted, linear or cyclo, alkyl, alkene, alkyne, aryl, alkylaryl, amine, hydroxyl, thiol, silyl, phosphoryl, cyano, metallocenyl, carbonyl, and polyphosphate.

4. A composition according to claim 1 wherein the underlying substrate is selected from the group consisting of a metal, a metal alloy, a polymer, a protein film, a glass or a ceramic.

5. A composition according to claim 1 wherein the second material is selected from the group consisting of inorganic crystalline structures, inorganic amorphous structures, organic crystalline structures, and organic amorphous structures.

6. A composition according to claim 5 wherein the second material is a phosphate.

7. A composition according to claim 5 wherein the second material is a calcium phosphate.

8. A composition according to claim 5 wherein the second material is selected from the group consisting of octacalcium phosphate, hydroxyapatite and carbonate apatite.

9. A composition according to claim 1 wherein the biologically active molecule is a protein, peptide, DNA segment, RNA segment, nucleotide, polynucleotide, nucleoside, antibiotic, antimicrobial, radioisotope, chelated radioisotope, chelated metal, metal salt, anti-inflammatory, steroid, nonsteroid anti-inflammatory, analgesic, antihistamine, receptor binding agent, or chemotherapeutic agent.

10. A composition according to claim 1 wherein the biologically active molecule is an osteogenic factor.

11. A composition of matter comprising a first porous metal material having a surface and attachment sites located on the surface and a self assembling mono layer containing functional groups provided at attachment sites, and a second crystallite calcium phosphate material nucleated by a functional group at an attachment site and grown as a dense film on the surface of the first material the calcium phosphate having an osteogenic factor incorporated therein.

12. A method for preparing a multi component coating which comprises treating a first material to produce a plurality of attachment sites on its surface, attaching a plurality of functional groups to the attachment sites, incorporating the functional groups into a thin-film of a second material deposited onto the material and providing a biologically active material which is incorporated onto or into the thin film deposited on the first material.

13. A method according to claim 12 wherein the treating step provides attachment sites to which a self assembling mono-layer can be attached.

14. A method according to claim 12 wherein the functional group attached to the attachment sites is chosen from the group consisting of carboxylates, sulfonates, phosphates, optionally substituted, linear or cyclo, alkyl, alkene, alkyne, aryl, alkylaryl, amine, hydroxyl, thiol, silyl, phosphoryl, cyano, metallocenyl, carbonyl, and polyphosphate.

15. A method according to claim 12 wherein the first material is selected from the group consisting of a metal, a metal alloy, a polymer, a protein film, a glass or a ceramic.

16. A method according to claim 12 wherein the second material is selected from the group consisting of inorganic crystalline structures, inorganic amorphous structures, organic crystalline structures, and organic amorphous structures.

17. A method according to claim 16 wherein the second material is a phosphate.

18. A method according to claim 16 wherein the second material is a calcium phosphate.

19. A method according to claim 16 wherein the biologically active molecule is a protein, peptide, DNA segment, RNA segment, nucleotide, polynucleotide, nucleoside, antibiotic, radioisotope, chelated radioisotope, chelated metal, metal salt, anti-inflamatory, steriod, nonsteriod anti-inflammatory, analgesic, antihistamine, receptor binding agent, or chemotherapeutic agent.

20. A method according to claim 16 wherein the biologically active molecule is an osteogenic factor.

21. An implant for use in a mammalian body comprising a composition according to claim 1.

22. A method for delivery of a biologically active substance which comprises treating an implant according to claim 12 and implanting the implant at a site to which the biologically active substance is to be delivered.

23. A bone fixation device comprising a composition according to claim 1.

24. A bone fixation device according to claim 23 wherein the biologically active molecule is an osteogenic factor.

25. A bone fixation device according to claim 23 wherein the first material is porous.

* * * * *

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